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The Danish contribution to the European DEMOCOPHES project: A description of cadmium, cotinine and mercury levels in Danish mother-child pairs and the perspectives of supplementary sampling and measurements

Thit A. Mørck [a], Flemming Nielsen [b], Jeanette K.S. Nielsen [a], Janne F. Jensen [a], Pernille W. Hansen [a], Anne K. Hansen [a], Lea N. Christoffersen [a], Volkert D. Siersma [c], Ida H. Larsen [a], Linette K. Hohlmann [a], Mette T. Skaanild [d], Hanne Frederiksen [e], Pierre Biot [f], Ludwine Casteleyn [g], Marike Kolossa-Gehring [h], Gerda Schwedler [h], Argelia Castaño [i], Jürgen Angerer [j], Holger M. Koch [j], Marta Esteban [i], Greet Schoeters [k], Elly Den Hond [k], Karen Exley [l], Ovnair Sepai [l], Louis Bloemen [m], Reinhard Joas [n], Anke Joas [n], Ulrike Fiddicke [h], Ana Lopez [i], Ana Cañas [i], Dominique Aerts [f], Lisbeth E. Knudsen [a,*]

[a] Department of Public Health, University of Copenhagen, Copenhagen, Denmark
[b] Department of Public Health, University of Southern Denmark, Odense, Denmark
[c] The Research Unit for General Practice and Section of General Practice, Department of Public Health, University of Copenhagen, Copenhagen, Denmark
[d] Danish Environmental Protection Agency, Denmark
[e] Department of Growth and Reproduction, University Hospital, Copenhagen, Denmark
[f] Federal Public Service Health, Food Chain Safety and Environment, Brussels, Belgium
[g] University of Leuven, Leuven, Belgium
[h] Federal Environment Agency (UBA), Berlin, Germany
[i] Instituto de Salud Carlos III (ISCIII), Majadahonda, Madrid, Spain
[j] Institute for Prevention and Occupational Medicine of the German social Accident Insurance, Institute of the Ruhr-Universität Bochum (IPA), Bochum, Germany
[k] Flemish Institute for Technological Research (VITO), Environmental Risk and Health unit, Mol, Belgium
[l] Public Health England, Centre for Radiation, Chemical and Environmental Hazards, Chilton, Oxfordshire, United Kingdom
[m] Environmental Health Science International, Hulst, The Netherlands
[n] BiPRO GmbH, Munich, Germany

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ABSTRACT

Human biomonitoring (HBM) is an important tool, increasingly used for measuring true levels of the body burdens of environmental chemicals in the general population. In Europe, a harmonized HBM program was needed to open the possibility to compare levels across borders. To explore the prospect of a harmonized European HBM project, DEMOCOPHES (DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale) was completed in 17 European countries. The basic measurements performed in all implemented countries of DEMOCOPHES included cadmium, cotinine and phthalate metabolites in urine and mercury in hair. In the Danish participants, significant correlations between mothers and children for mercury in hair and cotinine in urine were found. Mercury in hair was further significantly associated with intake of fish and area of residence. Cadmium was positively associated with BMI in mothers and an association between cadmium and cotinine was also found. As expected high cotinine levels were found in smoking mothers. For both mercury and cadmium significantly higher concentrations were found in the mothers compared to their children. In Denmark, the DEMOCOPHES project was co-financed by the Danish ministries of health, environment and food safety. The co-financing ministries agreed to finance a number of supplementary measurements of substances of current toxicological, public and regulatory interest. This also included blood sampling from the participants. The collected urine and blood samples were analyzed for a range of

* Corresponding author.
E-mail address: liek@sund.ku.dk (L.E. Knudsen).

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other persistent and non-persistent environmental chemicals as well as two biomarkers of effect. The variety of supplementary measurements gives the researchers further information on the exposure status of the participants and creates a basis for valuable knowledge on the pattern of exposure to various chemicals.

1. Introduction

In the last decades, human biomonitoring (HBM) has been increasingly contributing to policy legislation and regulations related to restrictions of environmental toxicants in consumer products and food. HBM can include both concentration measurements of pollutants in humans (biomarkers of exposure) and measurements of biological changes (biomarkers of effects) (Angerer et al., 2007). In Europe, several countries have national biomonitoring programs (Castaño et al., 2012; Cerna et al., 2012; Frery et al., 2012; Hohenblum et al., 2012; Kolossa-Gehring et al., 2012; Schoeters et al., 2012), however, a large program across European borders has been lacking. To improve and harmonize human biomonitoring (HBM) in Europe, the European pilot project DEMOCOPHES (DEMOnstration of a study to COordinate and Perform Human biomonitoring on a European Scale) was planned and performed in 17 European countries (Becker et al., 2014; Joas et al., 2012). The 17 countries participating were Denmark, Belgium, Cyprus, Czech Republic, Germany, Hungary, Ireland, Luxembourg, Poland, Portugal, Romania, Slovenia, Slovak Republic, Spain, Sweden, Switzerland and United Kingdom. Sampling was performed simultaneously in all countries from September 2011 to February 2012. The project involved collection of first morning urine void and hair sampling of school-children aged 6–11 years of age and their mothers. The mothers filled in a questionnaire regarding lifestyle, dietary habits and exposure risks. In the basic scenario of DEMOCOPHES, mercury was measured in hair, and cotinine, phthalate metabolites and cadmium were measured in the urine. Furthermore, creatinine was measured in urine and was used to adjust the aforementioned substances for urine concentration. In six countries, including Denmark, bisphenol A (BPA) was measured as an additional chemical in the urine (Covaci et al., in this issue). In Denmark the ministries of health, environment and food safety supporting and co-financing the DEMOCOPHES program in Denmark, requested and financed an expansion of the basic scenario. Additional measurements of substances of current toxicological, public and regulatory interest were therefore included in the Danish sub-study of DEMOCOPHES. The expansion of the scenario included blood sampling of the participants. This opportunity to measure a variety of chemicals in the same participants was unique and could be managed without much additional effort to the already planned collection of samples within the DEMOCOPHES project.

In the present paper, results from the measurements of mercury, cadmium and cotinine in the Danish DEMOCOPHES participants are reported in relation to lifestyle factors. Furthermore, the Danish results are compared to the total European DEMOCOPHES results. The extended scenario of the Danish DEMOCOPHES contribution is described and discussed with regards to future possibilities of HBM programs and enabling of more qualified risk assessment of exposure patterns of toxic environmental pollutants. Measurements of phthalate metabolites in the Danish DEMOCOPHES participants have been published previously together with the supplementary urinary measurements (Frederiksen et al., 2013b; Nielsen et al., 2014). Detailed results of the supplementary measurements in the blood samples are also described in individual publications (Mørck et al., 2014a; Mørck et al., 2014b).

2. Material and methods

2.1. Recruitment of study participants

The recruitment was performed in compliance with the COPHES protocol (Becker et al., 2014). In Denmark, the recruitment was accomplished via local schools in the selected areas. Schools were contacted and collaboration was established. Recruitment was carried out via the school intranet where an invitation to participate in the project was sent out to all parents of children in the age-group of 6 to 11 years of age. Information about the Danish DEMOCOPHES project was included in the e-mail and given orally at parent meetings. Mothers could sign up for participation and book an appointment for sampling on a project website, where they could also find additional information about DEMOCOPHES. To reach the required number of participants the sampling was expanded to include additional schools in each area and articles in local newspapers were used to increase local attention to the project. The two areas of recruitment in Denmark were Gentofte representing the urban area, and Viby Sjælland representing the rural area. Rural and urban areas were selected according to population density, where < 150 inhabitants/km^2 was defined as rural.

The following inclusion criteria were used: the child should be living with the mother a minimum of 16 days a month, the child and mother should have lived in the area for a minimum of 5 years, have sufficient Danish language knowledge and have normal kidney function and no metabolic disturbances. The goal of DEMOCOPHES was to reach 120 mother- child pairs in each country. In Denmark, 75 mother-child pairs from the urban area and 70 couples from the rural area were recruited, resulting in 145 mother-child pairs in total. The children were equally distributed in age, gender and urban/rural location. All participants received detailed written information about the study and gave informed consent before participating (for the child, all holders of custody should sign). The study was approved by the local regional ethics committee (H-3–2011-075) and the Danish data protection agency (2011-41-6607 and 2011-41-6766).

On the day of sampling, the completed questionnaire was handed in along with first morning urine samples. At the appointment hair samples were taken, and blood was drawn from the cubital vein. Mother-child pairs participating in a supplementary study on pain and self-medication were interviewed at the end of the visit. Sampling was conducted in parallel in the urban and the rural area from September to December 2011 to minimize seasonal variation.

2.2. Urine samples

Denmark participated in the development of the COPHES protocol, and the basic scenario chemicals for urine analysis (cotinine, cadmium and five phthalate metabolites as well as bisphenol A) were measured in laboratories which obtained successful results in the quality control program in DEMOCOPHES (Schindler et al., 2014, Esteban et al., in this issue)

The measurement of cotinine in urine was performed after solid phase extraction of the compound followed by quantitation on LC-MS/MS method with electrospray ionization (ESI) and single reaction monitoring (SRM) data acquisition (manuscript in prep). Creatinine was determined enzymatically on an Abbott Architect C8000 Clinical Chemistry Analyzer (Abbott Diagnostics, Lake Forrest, IL). The measurement of cadmium in urine was performed as previously described (Cañas et al., 2013). The urinary levels of cadmium and cotinine were adjusted for creatinine content in urine.

In Denmark the urinary measurements were expanded to include in total 15 phthalate metabolites (monoethyl phthalate (MEP), mono-iso-butyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), monobenzyl phthalate (MBzP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-n-octyl phthalate (MOP), mono(3-carboxypropyl) phthalate (MCPP), mono-iso-nonyl phthalate (MiNP), mono(hydroxy-iso-nonyl) phthalate (MHiNP), mono(oxo-iso-nonyl) phthalate (MOiNP), mono(carboxy-iso-octyl) phthalate (MCiOP) and mono-iso-decyl phthalate (MiDP)), seven parabens (methylparaben, ethylparaben, n-propylparaben, iso-propylparaben, iso-butylparaben, n-butylparaben and benzylparaben) and beside bisphenol A also eight other phenols (triclosan, triclocarban, benzophenone-3, 2,4-dichlorophenol, 2,5-dichlorophenol, 2,4,5-trichlorophenol, 2-phenylphenol and 4-phenylphenol). Description of the analysis was published elsewhere (Frederiksen et al., 2013a; Frederiksen et al., 2010, 2011; Frederiksen et al., 2013b). Finally, the urine samples were

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analyzed for the analgesic paracetamol, N-acetyl-4-aminophenol, which is published elsewhere (Nielsen et al., 2014).

The samples were collected following the protocol developed by the consortium (Becker et al., 2014). The laboratory analyses of the supplementary measurements were not subject to the same quality control as the compounds measured within all DEMOCOPHES countries (Schindler et al., 2014). They were, however, performed by the same certified laboratory at the Copenhagen University Hospital (Rigshospitalet, Copenhagen, Denmark) which also analyzed the samples for the phthalate metabolites included in DEMOCOPHES. For quality assurance the data was peer reviewed and published prior to feed back to the participants and the ministries (Frederiksen et al., 2013b). The paracetamol levels in urine were measured at Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (Institute of the Ruhr-Universität Bochum, Bochum, Germany) according to previously published methods (Modick et al., 2013; Dierkes et al. 2014).

2.3. Hair samples

Hair samples were analysed at the Department of Public Health, University of Southern Denmark, in a laboratory that participated and showed successful results in the hair analysis external quality assessment exercises within DEMOCOPHES (Esteban et al., in this issue). The measurement of mercury in hair was determined on a Flow Injection Mercury System 400 (Perkin Elmer, Waltham, MA) in the 3 cm of the hair closest to the scalp according to the method previously described (Grandjean et al., 1992).

2.4. Blood samples

On the day of sampling, blood was collected after handing in the questionnaire and urine samples. The venipunctures were performed by a trained biomedical laboratory technician, who was experienced in blood sampling from children. All children were offered local anesthetic cream prior to the puncture and it was explained that blood sampling was completely voluntary and that the participants could decline at any time. Approximately 20 mL blood was drawn in EDTA K3 plasma tubes for analysis of persistent organic pollutants (POPs) and centrifuged immediately for 10 min at 2000 g. Plasma was transferred to 5 ml PP cryo tubes and stored in a cooling box (4 °C) until arriving in the laboratory where it was stored at −20 °C until analysis. The samples were analysed for plasma concentrations of polychlorinated biphenyls (PCBs) (Mørck et al., 2014a), perfluoroalkyl substances (PFASs) (Mørck et al. 2014b) and polybrominated diphenyl ethers (PBDEs) (unpublished) at the University of Southern Denmark, which was also the DEMOCOPHES certified laboratory for mercury, cotinine and creatinine. 2 mL of blood was drawn in sodium heparin tubes for micronucleus (MN) analysis and stored in a cooling box. When arriving at the laboratory MN cell cultures were prepared from the blood. Micronuclei frequency in the lymphocytes was analyzed by Kim Vande Loock as described previously (Merlo et al., 2014; Vande Loock et al., 2011). Finally 10 mL blood was drawn in silica serum tubes for dioxin-like activity analysis. The tubes were left for one hour at room temperature to coagulate and then centrifuged for 10 min at 2000 g. Serum were transferred to Supelco tubes and stored in a cooling box until arriving at the laboratory where they were stored at −80 °C until further analysis at Aarhus University. The method is described in detail previously (Mørck et al., 2014a). In total, blood samples from 143 mothers and 123 children were collected, as the decline to venipuncture was respected. Not all samples from the children were complete due to insufficient blood flow.

2.5. Questionnaires

The basic questionnaire and the urine-sampling related questionnaire developed within the COPHES framework were translated into Danish to ensure that there was no language barrier. Questions regarding exposure relevant behavior related to the supplementary chemicals were included in the Danish questionnaire as part of the final questionnaire sent out to the participants. The additional questions were designed to increase the gained information on living conditions of the participants in relation to traffic exposure, as well as more detailed dietary exposure and use of specific personal care products such as sunscreen and antibacterial products. The questionnaires were filled in at home and handed in at the day of the sampling with an on-site dialog on potential non-response questions which were then resolved. Hereafter the blood sampling was performed and the participants were asked to participate in a supplementary study regarding pain and medicine use. This was described in detail previously (Jensen et al., 2014). All parents receiving the invitation to participate, but did not wish to take part in the DEMOCOPHES study, were encouraged to fill in a non-responder questionnaire which was located at the website for registration.

2.6. Feedback of results to study participants

After results were obtained for the measured chemicals, the Danish and European average results were sent out to the participants by mail. The material also included the results of the supplementary urine measurements. The participants were invited to an information meeting, where the results were explained, and the participants had the opportunity to ask questions. The letter also included a response letter including a few additional questions as well as a request for their personal Danish registration number (CPR) and an acceptance form, where permission could be given to follow-up in future studies. Finally, a form to fill in if participants wished to know their individual results was included. If this was the case, a new letter with individual results of the urine and hair analyses was sent to the requesting participants.

2.7. Statistics

In the present paper, statistical analysis was performed only on the data from the basic scenario. Differences between the Danish and European levels of cadmium, cotinine and mercury were tested by means of ANOVA. Correlations between the chemical levels in mothers and children and the concentrations in relation to age were tested by Spearman's rho. Differences between mothers and their children were assessed by Wilcoxon matched-pair signed-rank test. Associations of the levels of mercury in hair and cadmium in urine with relevant questions from the basic questionnaire were analyzed with logistic regression. The mercury and cadmium concentrations were dichotomized according to the median (0.27 μg/g hair in children and 0.44 μg/g hair in mothers) and the 75th percentile (0.04 μg/g creatinine for children and 0.2 μg/g creatinine for mothers), respectively. Samples below LOD were given the value of 0.5 LOD. For mercury the regression model included area, consumption of fish, saltwater fish, shellfish, freshwater fish and other seafood, BMI and level of education. Confounders adjusted for in children included age, gender, object containing mercury ever broken in house, language and mercury in mothers. For mothers confounders included age, amalgam fillings, use of make-up and eye make-up and object containing mercury ever broken in house. For cadmium the regression model included area, consumption of fish, shellfish, freshwater fish, rice, offal, meat, cereal, home grown food, level of education and BMI. The model for children was additionally adjusted for age, gender, smoking status of mother, passive smoking at home, painting at home within the last 4 weeks, source of drinking water, language, source of heating for cooking and mothers cadmium level. Confounders in the mothers were age, smoking status, passive smoking, painting at home, source of drinking water and source of heating for cooking. All statistical analyses were performed in IBM SPSS statistics version 20.

3. Results and discussion

The basic characteristics of the Danish DEMOCOPHES participants are shown in Table 1. In Denmark, 75 mother-child pairs from the urban area and 70 mother-child pairs from the rural area were recruited. The children were equally distributed according to age-group, gender and area. After feedback of results, 77% returned the additional questionnaire and of these, two mothers and eight children did not wish to give their personal registration number for future reference. Four mothers did not wish to receive the individual results. Among the recruited participants, 143 (99%) mothers and 123 (85%) children donated blood samples. The distribution of blood donation according to age and gender can be seen in Fig. 1. Younger children were more likely not to donate blood, and this was not dependent on gender. Reasons for not donating blood samples were simple decline, dizziness, failure of venipuncture/drawing of blood and fear of the needle among children.

The main difference between the protocol developed within the COPHES framework and the protocol used in the Danish part of DEMOCOPHES was the addition of the blood sampling. We also chose self-registration online, thus depending on that mothers take the initiative and sign up via the internet. We did not contact potential participants personally by mail or telephone calls, however e-mail invitations were sent out to potential participants via the schools intranet. This turned out very successful for recruitment in the particular target group and we ended up with 145 mother-child couples, thus 25 more than the minimum

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Table 1
Characteristics of the Danish DEMOCOPHES participants including feedback results and request of personal results.

	Children			Mothers		
	urban	rural	all	urban	rural	all
n	75	70	145	75	70	145
Age (years) (Mean)	8.5 (6–11)	8.5 (6–11)	8.5 (6–11)	41.9 (31–52)	39.9 (31–50)	40,9 (31–52)
Sex (*n*)	35♂, 40♀	35♂, 35♀	70♂, 75♀	–	–	–
BMI (body mass index) (mean)	15.8 ± 1.2	16.6 ± 2.33	16.2 ± 2.3	22.1 ± 2.7	24.8 ± 5.7	23.4 ± 4.6
Blood samples (*n*)	61	62	123	73	70	143
Feed back results (no CPR**) (*n*)	62 (5)	50 (3)	112	62 (1)	50 (1)	112
Requested personal data (*n*)	–	–	–	73	68	141
Fish consumption* (*n*)						
Several times a week	27	29	57	43	21	64
once a week	41	35	76	16	22	38
less than once a week	7	6	13	16	27	43
Saltwater fish (*n*)						
Weekly	36	27	73	36	32	68
monthly	32	29	61	35	27	62
Less than once a month	7	14	21	4	10	14
Smokers (*n*)	–	–	–	6	12	18
Rice (*n*)						
Often	32	22	54	52	50	102
Almost never	43	48	91	23	19	42

* Total consumption of fish (saltwater fish, freshwater fish, shellfish and other food from the sea or lake).
** CPR is the Danish registration number. Not all participants agreed to give their CPR for follow up.

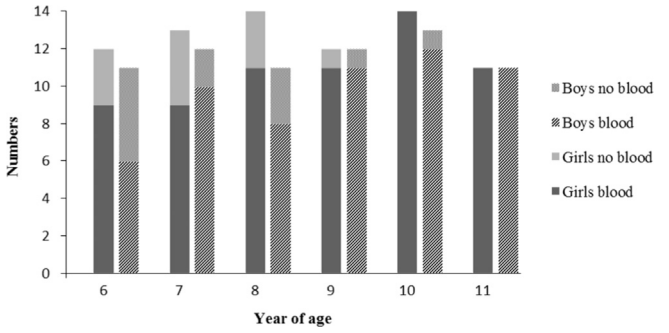

Fig. 1. Number of blood samples from children distributed in age and gender. Younger children were more likely not to donate blood samples. There was no difference in gender.

requirements of 120 set by DEMOCOPHES framework. The contact via school intranet and press announcements in local newspapers of the DEMOCOPHES project was reliant on active enrollment and consequently resulted in a low response-rate on the non-responder questionnaire. Only seventeen mothers completed the non-responder questionnaire, with several different answers for not participating. This questionnaire could therefore not be used for any conclusions regarding not wishing to or being able to participate in the study.

The internet recruitment and active enrollment may result in bias regarding the socioeconomic status of the participants as well as recruitment of already health enthusiastic participants. However, in Denmark it is most common to use the internet as a platform for information and school-parent communication. Therefore this was an obvious and effective way to contact potential participants.

3.1. Ethics

The project was approved by the the local regional ethics committee and the Danish data protection agency (see 2.1). During the sample collection special attention was given to the blood sampling of the children. Blood was drawn by experienced technicians and great care was taken to give the children the best possible experience. If there was any kind of discomfort, the procedure was stopped immediately and the child was taken care of. This resulted in a less complete set of blood samples compared to the urine samples; however, the well-being and emotional experience of the children was of highest priority. Furthermore, some blood samples from the children did not contain sufficient blood for analysis of all supplementary blood measurements. This explains why the PCBs are measured in 116 children whereas for example dioxin like activity is measured in 120 children (see Table 1).

3.2. Results from the basic DEMOCOPHES scenario in Denmark

The geometric means and 95% confidence interval of mercury, cadmium and cotinine of the Danish mothers and children with comparative values for the European study are shown in Table 2.

3.2.1. Mercury

Mercury was found in the hair of almost all Danish participants (mothers 98.6% and children 97.9%, see Table 3) and ranged from below limit of detection (LOD) (0.04 μg/g) to 2.822 μg/g hair in mothers and < LOD to 1.335 μg/g hair in children. The geometric mean (GM) was 0.249 μg/g in children and 0.420 μg/g in mothers, the mercury in mothers being significantly higher compared to the children. Furthermore, for the mothers there was a significant correlation between age and mercury level ($\rho = 0.247$, $p < 0.01$). The increase in mercury with age has also been found in other studies (Chien et al., 2010; Dorea et al., 2003) and is presumably caused by the cumulate nature of mercury. A significant correlation between the mercury hair levels in mothers and children was also found (Spearman's rho $\rho = 0.509$, $p < 0.001$), indicating family related exposure sources.

The results of the statistical analysis of the Danish measurements of mercury in hair in relation to lifestyle factors are shown in Table 4. Analysis of logistic regression showed that area of residence is the most predictive factor of the mercury level in hair in the mothers, also

Table 2
Concentration (geometric means) and 95% confidence intervals (in parenthesis) of mercury in hair and urinary cotinine and cadmium in mothers and children.

	LOD[a]	Denmark			DEMOCOPHES			ANOVA*
		urban	rural	all	urban	rural	all	*P*-value
Children								
Mercury (μg/g hair)	0.04 μg/g	0.283 (0.237–0.339) ($n=74$)	0.217 (0.181–0.262) ($n=70$)	0.249 (0.219–0.284) ($n=144$)	0.174 (0.160–0.189) ($n=916$)	0.117 (0.108–0.128) ($n=920$)	0.143 (0.135–0.339) ($n=1836$)	**< 0.001**
Cadmium (μg/L urine)	0.004 μg/l	0.019 (0.016–0.023) ($n=74$)	0.024 (0.019–0.029) ($n=68$)	0.021 (0.019–0.024) (142)	0.066 (0.062–0.070) ($n=848$)	0.069 (0.065–0.074) ($n=850$)	0.067 (0.064–0.071) ($n=1698$)	**< 0.001**
Cadmium (μg/g creatinine)	–	0.020 (0.017–0.024) ($n=74$)	0.025 (0.020–0.032) ($n=68$)	0.022 (0.020–0.026) ($n=142$)	0.062 (0.058–0.066) ($n=848$)	0.068 (0.064–0.073) ($n=850$)	0.065 (0.062–0.068) ($n=1698$)	**< 0.001**
Cotinine (μg/L urine)	0.3 μg/l	0.570 (0.484–0.671) ($n=74$)	0.745 (0.591–0.940) ($n=70$)	0.649 (0.563–0.748) ($n=144$)	0.793 (0.734–0.857) ($n=906$)	0.839 (0.770–0.915) ($n=912$)	0.816 (0.770–0.865) ($n=1818$)	**0.03**
Cotinine (μg/g creatinine)	–	0.590 (0.483–0.721) ($n=74$)	0.788 (0.629–0.988) ($n=70$)	0.679 (0.584–0.791) ($n=144$)	0.749 (0.629–0.988) ($n=906$)	0.824 (0.754–0.900) ($n=912$)	0.785 (0.740–0.834) ($n=1818$)	0.2
Mothers								
Mercury (μg/g hair)	0.04 μg/g	0.548 (0.454–0.660) ($n=75$)	0.316 (0.269–0.372) ($n=70$)	0.420 (0.368–0.479) ($n=145$)	0.283 (0.261–0.307) ($n=917$)	0.187 (0.172–0.203) ($n=922$)	0.230 (0.217–0.244) ($n=1839$)	**< 0.001**
Cadmium (μg/L urine)	0.004 μg/l	0.103 (0.082–0.130) ($n=74$)	0.130 (0.105–0.160) ($n=68$)	0.115 (0.098–0.135) ($n=142$)	0.216 (0.203–0.229) ($n=844$)	0.220 (0.207–0.233) ($n=841$)	0.218 (0.209–0.227) ($n=1685$)	**< 0.001**
Cadmium (μg/g creatinine)	–	0.109 (0.089–0.134) ($n=74$)	0.129 (0.109–0.151) ($n=68$)	0.118 (0.104–0.135) ($n=142$)	0.195 (0.185–0.204) ($n=844$)	0.192 (0.183–0.202) ($n=841$)	0.193 (0.187–0.200) ($n=1685$)	**< 0.001**
Cotinine (μg/L urine)	0.3 μg/l	1.456 (0.855–2.480) ($n=74$)	1.634 (0.886–3.015) ($n=69$)	1.539 (1.029–2.303) ($n=143$)	2.420 (1.998–2.930) ($n=902$)	3.598 (2.908–4.452) ($n=898$)	2.950 (2.556–3.404) ($n=1800$)	**0.03**
Cotinine (μg/g creatinine)	–	1.542 (0.886–2.685) ($n=74$)	1.602 (0.845–3.038) ($n=69$)	1.571 (1.032–2.391) ($n=0143$)	2.183 (1.805–2.640) ($n=902$)	3.144 (2.543–3.887) ($n=898$)	2.619 (2.271–3.020) ($n=1800$)	0.09

* Test of differences between the Danish (all) and European concentrations by means of ANOVA. *P*-values marked in bold are below significance level of 0.05.
[a] LOD = limit of detection. Samples below LOD was given the value of 1/2 LOD.

after adjusting for age, amalgam fillings, language, use of make-up and mercury-containing broken object. Mothers living in the urban area had a 5 times higher risk of having a high mercury level above set threshold (Table 4). Mercury levels were also positively associated with consumption of saltwater fish among the mothers, with mothers eating saltwater fish weekly having a 3 times higher risk of a high mercury concentration in hair (Table 4). Positive correlation was also observed between mercury level and overall fish consumption (saltwater fish, freshwater fish, shellfish and other seafood) in the children, however with a less clear picture since children eating fish weekly actually had a higher risk of a high mercury concentration compared to children eating fish daily. The lowest risk was, however, still found in children eating fish less than weekly (Table 4). The association with increased intake of fish and higher levels of mercury in hair is well established (Björnberg et al., 2005; Jenssen et al., 2012; Passos et al., 2007) and was also present in the total DEMOCOPHES population (Den Hond et al., 2014 submitted for publication). As can be seen in Table 1, mothers in the urban area eat fish more frequently compared to mothers in the rural area. This may explain the higher levels of hair mercury found in the urban mothers. Urban and rural children have similar frequencies of fish consumption, which may explain why we do not find significant differences in child mercury levels between the living areas.

The mercury concentrations in hair of the Danish mothers and children were significantly higher compared to the average European level (Table 2), possibly due to the relatively high consumption of fish in Denmark, compared to the other participating countries (Den Hond et al., 2014 submitted for publication; Castaño et al., in this issue).

3.2.2. Cadmium

Cadmium was detected in 32.6% of the children and 91.6% of the mothers (Table 3). The geometric mean (GM) of cadmium in children was 0.021 μg/L and levels ranged from < LOD (0.004 μg/L) to 0.27 μg/L. In mothers the concentrations were significantly higher with a GM of 0.115 μg/L and range from < LOD to 1.09 μg/L (see Table 2). Also in mothers, a positive correlation with cadmium levels adjusted for creatinine and age was found ($\rho=0.256$, $p<0.01$). Cadmium accumulates in the human body and the increase in urinary cadmium levels with age has been found consistently (Nikic et al., 2005; Olsson et al., 2002; Pirard et al., 2014; Richter et al., 2009) and was also seen in the total European DEMOCOPHES population (Den Hond et al., 2014 submitted for publication). There was no significant correlation between the cadmium levels in mothers and children. Furthermore, smoking mothers had significantly higher levels of cadmium in the urine ($p<0.001$) compared to non-smoking mothers. Increased levels of cadmium in the urine of smokers is also well established (Jarup, 2003). A significant correlation between cadmium and cotinine was found in the present data for both mothers ($\rho=0.214$, $p<0.05$) and children ($\rho=0.208$, $p<0.05$) for creatinine adjusted concentrations. After adjusting for possible confounders the best predictor for high levels of cadmium in the urine of the Danish mothers was BMI (Table 5). However, a negative association between consumption of meat and high cadmium levels was also found (see Table 5). The association between BMI and cadmium may be due to the fact that people with higher BMI consume more food and hence increase their exposure to cadmium as the main route of exposure is through the diet. For children a negative relation between consumption of cereal and belonging to the group with highest cadmium level was found. While a positive, however statistically insignificant correlation between consumption of rice and high cadmium levels in urine was seen (see Table 5). The negative relationship between cereal consumption and cadmium is opposite of what would be expected as cereal is

Table 3
Detection frequencies of the chemical compounds or biomarkers measured in the participants in percent.

Urine

Phthalate metabolites	LOD (ng/mL)	Mother ($n=145$)	Child ($n=143$)	**Parabens**	LOD (ng/mL)	Mother ($n=145$)	Child ($n=143$)	**Phenols**	LOD (ng/mL)	Mother ($n=145$)	Child ($n=143$)	Others	LOD (ng/mL)	Mother	Child
MEP	0.53	100	100	Methylparaben	0.26	90	63	**Bisphenol A**	0.12	97	93	**Cadmium**	0.004	92 ($n=142$)	33 ($n=141$)
MiBP	1.10	100	100	Ethylparaben	0.40	66	50	Triclosan	0.06	80	79	**Cotinine**	0.3	54 ($n=143$)	40 ($n=143$)
MnBP	1.43	100	100	n-Propylparaben	0.18	15	3	Triclocarban	0.01	25	28				
MBzP	1.14	92	97	i-Propylparaben	0.18	83	46	Benzophenon-3	0.07	98	97	Paracetamol	0.25	100 ($n=145$)	99.3 ($n=145$)
MEHP	0.14	91	92	i-butylparaben	0.07	0	0	2,4-dichlorophenol	0.07	86	93				
MEHHP	0.91	100	100	n-butylparaben	0.07	39	17	2,5-dichlorophenol	0.07	81	85				
MEOHP	0.67	99	100	Benzylparaben	0.18	2	4	2,4,5-trichlorophenol	0.06	14	11				
MECPP	0.55	100	100					2-phenylphenol	0.12	52	56				
MOP	0.15	5	14					4-phenylphenol	0.13	48	38				
MCPP	0.36	99	100												
MiNP	0.61	15	17									**Hair**	µg/g		
MHiNP	0.26	97	100											Mother ($n=145$)	Child ($n=144$)
MOiNP	0.25	90	99												
MCiOP	0.11	100	100									**Mercury**	0.04	99	98
MiDP	0.69	0	0												

Blood

PCBs	LOD (ng/mL)	Mother ($n=143$)	Child ($n=116$)	**PFASs**	LOD (pg/ml)	Mother ($n=143$)	Child ($n=118$)	**PBDE**	LOD (pg/ml)	Mother ($n=143$)	Child ($n=118$)	**Biomarkers**	Mother	Child
PCB-28	0.02	56	81	PFOA	0.03	100	100	PBDE-28	0.20	100	100	MN	*($n=119$)	*($n=103$)
PCB-52	0.02	1.4	7.8	PFHxS	0.03	100	99	PBDE-47	0.25	100	100	AhR-TEQ	65 ($n=143$)	56 ($n=120$)
PCB-101	0.02	1.4	2.6	PFNA	0.03	100	100	PBDE-99	0.50	100	100			
PCB-105	0.02	3.5	3.4	PFDA	0.03	100	100	PBDE-100	0.25	100	100			
PCB-118	0.02	79	43	*br*-PFOS	0.03	100	100	PBDE-153	1.00	100	100			
PCB-138	0.02	100	100	*n*-PFOS	0.03	100	100	PBDE-154	1.00	36	59			
PCB-153	0.02	100	99					PBDE-183	2.5	4.2	2.5			
PCB-156	0.02	83	49											
PCB-180	0.02	100	99											
β-HCH	0.02	61	22											
HCB	0.02	100	100											
o,p′–DDT	0.02	0.7	1.7											
*p,p′*DDT	0.02	64	54											
o,p′–DDE	0.02	0	0											
p,p′–DDE	0.02	100	100											

Metabolites marked in bold was included in the basic scenario of DEMOCOPHES.

* Numbers of slides

Table 4
The risk of having a mercury level above median in children (0.25 μg/g hair) and mothers (0.42 μg/g hair) due to listed exposures.

Variables		Children									Mothers								
		Effect of individually exposure*					Effect of all exposures**				Effect of individually exposure*					Effect of all exposures**			
		n	OR	95% CI	*P*-value[a]	Trend[b]	OR	95% CI	*P*-value[a]	Trend[b]	*n*	OR	95% CI	*P*-value[a]	Trend[b]	OR	95% CI	*P*-value[a]	Trend[b]
Area	Urban	74	1								75	1				1			
	Rural	70	0.51	0.21;1.23	0.132						70	0.20	0.08;0.49	**0.000**		0.19	0.09;0.41	**0.000**	
Fish Consumption	Daily	55	1		(0.082)		1		**(0.014)**		64	1		**(0.005)**					
	Weekly	76	3.03	1.12;8.17	**0.028**	0.170	2.66	1.18;6.01	0.018	0.524	38	0.25	0.09;0.70	0.008	**0.003**				
	Less than weekly	13	1.39	0.26;7.26	0.700		0.54	0.13;2.29	0.403		43	0.22	0.08;0.62	0.004					
Saltwater fish	Weekly	63	1		(0.271)						68	1		**(0.048)**		1		**(0.013)**	
	Monthly	60	1.05	0.41;2.71	0.922	0.244					62	0.34	0.15;0.80	0.014	0.053	0.31	0.14;0.68	0.004	**0.016**
	Less than monthly	21	0.34	0.08;1.43	0.140						14	0.58	0.12;2.81	0.502		0.45	0.12;1.63	0.225	
Shellfish	Weekly	11	1		(0.428)						33	1		(0.450)					
	Monthly	59	0.51	0.10;2.52	0.409	0.207					84	1.43	0.53;3.82	0.478	0.688				
	Less than monthly	72	0.37	0.08;1.77	0.214						27	0.77	0.23;2.58	0.665					
Freshwater fish	Weekly	10	1		(0.177)						13	1		(0.077)					
	Monthly	38	0.17	0.01;2.09	0.166	0.071					45	0.14	0.02;0.80	0.027	0.465				
	Less than monthly	92	0.11	0.01;1.28	0.078						85	0.24	0.05;1.31	0.100					
Other seafood	Several times a month	13	1		(0.637)						18	1		(0.129)					
	Once a month	20	0.70	0.09;5.46	0.737	0.343					33	0.35	0.07;1.79	0.206	**0.045**				
	Less than monthly	108	0.49	0.09;2.74	0.412						90	0.22	0.05;1.01	0.051					
Tertiary education	No	10	1								17	1							
	Yes	134	0.49	0.10;2.49	0.387						127	2.70	0.73;9.99	0.138					
BMI (kg/m^2)	< 17	107	1								108	1							
	> 17	35	0.61	0.21;1.77	0.363						36	0.52	0.19;1.42	0.202					

OR=Odds ratio, CI=Confidence interval.

* Adjusted for childs age and gender, household object containing Hg ever broken in the house, language and mothers Hg.

** Adjusted for mothers mercury ($p=0.000$).

[a] *P*-values in brackets (*P*-value) are for over all tests.

[b] *P*-value for test for trend.

Table 5
The risk of having a cadmium level above 75 percentile in children (0.04 μg/g) and mothers (0.2 μg/g) due to the listed exposures.

Variables		Children									Mothers								
		Effect of individually exposure*					Effect of all exposures**				Effect of individually exposure*					Effect of all exposures**			
		n	OR	95% CI	*P*-value[a]	Trend[b]	OR	95% CI	*P*-value[a]	Trend[b]	n	OR	95% CI	*p*-value[a]	Trend[b]	OR	95% CI	*P*-value[a]	Trend[b]
Area	Urban	74	1				1				74	1							
	Rural	68	2.27	0.85;6.08	0.102		2.91	1.19;7.15	**0.020**		68	1.62	0.59;4.46	(0.353)					
Fish Consumption	Daily	55	1		(0.743)						63	1		0.204					
	Weekly	75	0.70	0.28;1.74	0.441	0.570					37	1.36	0.43;4.26	0.601	0.081				
	Less than weekly	12	0.84	0.16;4.37	0.839						42	2.56	0.90;7.31	0.079					
Freswater fish	Weekly	10	1		(0.125)		1		**(0.044)**		12	1		(0.218)					
	Monthly	37	0.78	0.13;4.68	0.787	**0.050**	0.88	0.16;4.90	0.884	**0.018**	45	5.94	0.79;44.62	0.083	**0.531**				
	Less than monthly	91	0.33	0.06;1.82	0.202		0.28	0.05;1.50	0.137		83	3.74	0.58;23.95	0.164					
Shellfish	Weekly	10	1		(0.278)						32	1		(0.277)					
	Monthly	59	3.02	0.30;30.10	0.345	0.108					83	1.43	0.45;4.48	0.544	0.132				
	Less than monthly	71	4.94	0.50;48.99	0.172						26	3.16	0.74;13.53	0.121					
Rice	Often	53	1				1				99	1							
	Rarely	89	0.50	0.21;1.19	0.118		0.42	0.17;1.01	**0.052**		42	0.67	0.25;1.79	0.426					
Offal	Yes	20	1								25	1							
	No	121	0.68	0.22;2.09	0.500						115	1.28	0.40;4.08	0.676					
Meat	Daily	97	1								77	1				1			
	Less than daily	45	0.58	0.22;1.54	0.273						64	2.82	1.15;6.92	0.024		3.53	1.34;9.26	**0.010**	
Cereal	Often	121	1				1				104	1							
	Rarely	20	2.93	0.99;8.65	0.052		3.77	1.22;11.63	**0.021**		37	0.51	0.18;1.47	0.212					
Homegrown Food	Often	46	1								50	1				1			
	Rarely	96	0.90	0.36;2.23	0.823						91	2.47	0.90;6.79	0.080		2.54	0.89;7.26	0.082	
Tertiary Education	No	10	1								16	1							
	Yes	132	1.08	0.17;6.92	0.934						125	0.62	0.16;2.43	0.489					
BMI (kg/m^2)	< 17	105	1								106	1				1			
	> 17	34	0.65	0.23;1.83	0.411						35	4.58	1.56;13.42	0.006		6.36	2.08;19.41	**0.001**	

OR=Odds ratio, CI=Confidence interval.

* Adjusted for childs age and gender, mothers smoking status, passive smoking at home, language, anyone working with paintings/at home within the last four weeks, source of drinking water, use of gas as main source of heating for cooking, mothers Cd (75 percentile).

** Adjusted for working with painting/coating in home within the last 4 weeks ($p=0.055$).

[a] *P*-values in brackets (*P*-value) are for over all tests.

[b] *P*-value for test for trend

one of the known sources of cadmium. This discrepancy may be due to no spatial differences exist.

Furthermore, the children in the rural area was of almost 3 times higher (see Table 5) risk at having a cadmium level in the high level group. This was opposite of what was found with mercury in the mothers, where mercury was higher in the urban area. We have also investigated the area differences in the non-persistent chemicals, where we found no differences, except for benzophenone-3 and some parabens, which were higher in the urban area (Frederiksen et al., 2013b). For the POPs, no differences exists in the level of PFASs, however several of the PCBs were also higher in the urban area, as were the dioxin like activity (Mørck et al., 2014a) and MN in mothers. We have found no clear source of the differences of levels for some of the measured substances; however, the consumption of fish may be an explanation also for the higher levels of PCBs and dioxin-like activity in urban families.

The cadmium levels in the Danish mothers and children are significantly lower compared to the European levels in both rural and urban areas as well as the total levels (Table 2).

3.2.3. Cotinine

Cotinine in urine was found in 39.9% of the children and 53.8% of the mothers (Table 2). High cotinine (>200 μg/L) was found in all mothers who reported smoking. Three mothers reporting to be non-smokers had high levels of cotinine, but were exposed to second hand smoke often or used nicotine gum. Children of smoking mothers and children exposed to other second hand smoke had elevated levels of cotinine in the urine, however the highest measured cotinine level in the children was 16.3 μg/L compared to a maximum level of 3403 μg/L in the mothers. The Spearman correlation between cotinine in mothers and children was significant for both creatinine adjusted ($\rho=0.408$, $p<0.001$) and non-adjusted ($\rho=0.528$, $p<0.001$) cotinine levels.

3.3. Supplementary biomarker measurements in Denmark

The goal of the supplementary measurements was to increase the knowledge of the exposure of the Danish population to potential endocrine disrupting chemicals and to explore the opportunity to envision a pattern of exposure to a large number of both persistent and non-persistent chemicals. The urinary analysis was expanded to include seven parabens, nine phenols, additional phthalate metabolites (Frederiksen et al., 2013b) and paracetamol (Nielsen et al., 2014). National attention was also given to persistent organic pollutants (POPs), which can be measured in blood samples. For information about general population levels in Denmark the ministries supported the analysis of PCBs (Mørck et al., 2014a), PBDEs (unpublished) and PFASs (Mørck et al. 2014b). The exposure to these compounds was only scarcely analyzed in Denmark before, while the exposure to the non-persistent chemicals has previously been analyzed in other segments of the Danish population (Frederiksen et al., 2014).

Throughout the development of the COPHES project much interest was also expressed towards biomarkers of effect and as part of a Danish PhD program analyses of the AhR-TEQ (TCDD toxic equivalent) in serum samples processed for the analysis of hormone activity in an in vitro assay (Mørck et al., 2014a) were included in the study protocol. The blood samples were also analyzed for frequency of micronuclei (unpublished data) performed in collaboration with the group of Micheline Kirsch-Volders in Belgium as in previous programs (Merlo et al., 2014; Vande Loock et al., 2011).

The availability of analytical methods for supplementary measurements at the two analytical facilities enabled our extended program as financial support was also provided from the ministries. Furthermore, the PhD grant from the University of Copenhagen, Faculty of Health and Medical sciences to Thit Aarøe Mørck enabled data processing and publishing. The expanded Danish analysis program provided a combined HBM study with more specific questions and more chemicals analyzed. Combined analyses provided more possibilities for identifying consumer sources for human exposure. Finally the blood and urine samples collected within few hours provide possibilities for additional analysis of metabolism and distribution of chemicals and as well as a range of further analyses now and in future. All substances measured in the Danish DEMOCOPHES participants are summarized in Table 3.

In total concentrations of 63 chemicals/metabolites and two biomarkers of effects were measured in the Danish DEMOCOPHES participants (Table 3). Many of the measured chemicals were detected in the majority of the participants, which clearly shows that both mothers and children are exposed to numerous different chemicals simultaneously. This emphasizes the importance of including as many analytes concurrently in HBM studies as possible. Because the DEMOCOPHES laboratories in Denmark were able to perform validated analysis of the chosen supplementary chemicals and due to willingness of financial support by the Danish ministries, much more information on the exposure status of the participants was gained, without new recruiting programs. This enables a unique comparison of the exposure to different chemicals one may not have combined elsewhere, as well a possible disclosure of exposure patterns related to certain lifestyles or behavior. Furthermore the information of exposure level may be used in risk assessment of combined or mixed exposure, which is of increasing interest because of possible combination effects of exposure to many chemicals with the same endogen target or effect at the same time.

4. Conclusion

Results from the Danish basic DEMOCOPHES measurements of mercury, cadmium and cotinine showed that the Danish participants are exposed to higher levels of mercury and lower levels of cadmium compared to the European DEMOCOPHES study population. In Denmark, mercury levels are related to fish consumption, age and area of residence, whereas cadmium is related to age and cigarette smoking in mothers. High levels of cotinine were almost only observed in smokers, as expected. The inclusion of supplementary measurements in the Danish DEMOCOPHES study complied with the basic protocol and has resulted in a great range of possibilities for gaining more knowledge of the exposure to many different substances of toxicological interest at the same time. The expanded urinary measurements and additional analyses of blood samples provided the possibility to examine the exposure patterns related to lifestyle in a group of Danish mother-child pairs. The inclusion of measurements of many substances of interest at the same time using several matrices from the same person is strongly recommended for future HBM programs. It creates a unique opportunity to find exposure patterns in lifestyle, living area or behavioral factors that may provide new knowledge on exposure to many different substances simultaneously. This may also be important in future risk assessment and evaluations on mixtures of chemicals.

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For information on both projects as well as the national co-funding institutions please visit the webpage: http://www.eu-hbm.info/.

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References

Angerer, J., Ewers, U., Wilhelm, M., 2007. Human biomonitoring: state of the art. Int. J. Hyg. Environ. Health 210, 201–228.

Becker, K., Seiwert, M., Casteleyn, L., Joas, R., Joas, A., Biot, P., Aerts, D., Castaño, A., Esteban, M., Angerer, J., et al., 2014. A systematic approach for designing a HBM pilot study for Europe. Int. J. Hyg. Environ. Health 217, 312–322.

Björnberg, K.A., Vahter, M., Grawé, K.P., Berglund, M., 2005. Methyl mercury exposure in Swedish women with high fish consumption. Sci. Total Environ. 341, 45–52.

Cañas, A.I., Esteban, M., Cutanda, F., Castaño, A., 2013. Risk of overestimation of urinary cadmium concentrations: interference from molybdenum. E3S Web of Conferences 1, 21003. http://dx.doi.org/10.1051/e3sconf/20130121003.

Castaño, A., Sanchez-Rodriguez, J.E., Canas, A., Esteban, M., Navarro, C., Rodriguez-Garcia, A.C., Arribas, M., Diaz, G., Jimenez-Guerrero, J.A., 2012. Mercury, lead and cadmium levels in the urine of 170 Spanish adults: a pilot human biomonitoring study. Int. J. Hyg. Environ. Health 215, 191–195.

Castaño, A., Cutanda, F., Esteban, M., Navarro, C., Part, P., Gomez, S., López, A., Rosado, M., Posada, M., López, E., et al. in this issue. Fish patterns and hair mercury levels in children and their mothers of 17 European countries.

Cerna, M., Krskova, A., Cejchanova, M., Spevackova, V., 2012. Human biomonitoring in the Czech Republic: an overview. Int. J. Hyg. Environ. Health 215, 109–119.

Chien, L.C., Gao, C.S., Lin, H.H., 2010. Hair mercury concentration and fish consumption: risk and perceptions of risk among women of childbearing age. Environ. Res. 110, 123–129.

Covaci, A., Den Hond, E., Geens, T., Govarts, E., Koppen, G., Frederiksen, H., Knudsen, L.E., Mørck, T.A., Gutleb, A.C., Guignard, C., et al. in this issue. Urinary BPA measurements in children and mothers from six European member states: overall results and determinants of exposure.

Den Hond, E., Govarts., E., Willems, H., Smoelders, R., Casteleyn, L., Kolossa-Gehring, M., Schwedler, G., Seiwert, M., Fiddicke, U., Castaño, A., et al. 2014 submitted for publication. The first steps towards harmonised human biomonitoring in Europe.

Dierkes, G., Weiss, T., Modick, H., Kafferlein, H.U., Bruning, T., Koch, H.M., 2014. N-Acetyl-4-aminophenol (paracetamol), N-acetyl-2-aminophenol and acetanilide in urine samples from the general population, individuals exposed to aniline and paracetamol users. Int. J. Hyg. Environ. Health 217, 592–599.

Dorea, J., Barbosa, A.C., Ferrari, I., de Souza, J.R., 2003. Mercury in hair and in fish consumed by Riparian women of the Rio Negro, Amazon, Brazil. Int. J. Environ. Health Res. 13, 239–248.

Esteban, M., Schindler, B.K., Koch, H.M., Jiménez-Guerro, J.A., Angerer, J., Casteleyn, L., Becker, K., Kolossa-Gehring, M., Den Hond, E., Schoeters, G., et al. in this issue. Comparability and quality assess,ent within the European human biomonitoring pilot study - Mercury analysis in hair.

Frederiksen, H., Aksglaede, L., Sorensen, K., Nielsen, O., Main, K.M., Skakkebaek, N. E., Juul, A., Andersson, A.M., 2013a. Bisphenol A and other phenols in urine from Danish children and adolescents analyzed by isotope diluted TurboFlow-LC-MS/MS. Int. J. Hyg. Environ. Health 216, 710–720.

Frederiksen, H., Jensen, T.K., Jorgensen, N., Kyhl, H.B., Husby, S., Skakkebaek, N.E., Main, K.M., Juul, A., Andersson, A.M., 2014. Human urinary excretion of non-persistent environmental chemicals: an overview of Danish data collected between 2006 and 2012. Reproduction 147, 555–565.

Frederiksen, H., Jorgensen, N., Andersson, A.M., 2010. Correlations between phthalate metabolites in urine, serum, and seminal plasma from young Danish men determined by isotope dilution liquid chromatography tandem mass spectrometry. J. Anal. Toxicol. 34, 400–410.

Frederiksen, H., Jorgensen, N., Andersson, A.M., 2011. Parabens in urine, serum and seminal plasma from healthy Danish men determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). J. Expo. Sci. Environ. Epidemiol. 21, 262–271.

Frederiksen, H., Nielsen, J.K., Mørck, T.A., Hansen, P.W., Jensen, J.F., Nielsen, O., Andersson, A.M., Knudsen, L.E., 2013b. Urinary excretion of phthalate metabolites, phenols and parabens in rural and urban Danish mother-child pairs. Int. J. Hyg. Environ. Health 216, 772–783.

Frery, N., Vandentorren, S., Etchwevers, A., Fillol, C., 2012. Highlights of recent studies and future plans for the French human biomonitoring (HBM) programme. Int. J. Hyg. Environ. Health 215, 127–132.

Grandjean, P., Weihe, P., Jorgensen, P.J., Clarkson, T., Cernichiari, E., Videro, T., 1992. Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. Arch. Environ. Health 47, 185–195.

Hohenblum, P., Steinbichl, P., Raffesberg, W., Weiss, S., Moche, W., Vallant, B., Scharf, S., Haluza, D., Moshammer, H., Kundi, M., et al., 2012. Pollution gets personal! A first population-based human biomonitoring study in Austria. Int. J. Hyg. Environ. Health 215, 176–179.

Jarup, L., 2003. Hazards of heavy metal contamination. Br. Med. Bull. 68, 167–182.

Jensen, J.F., Gottschau, M., Siersma, V.D., Graungaard, A.H., Holstein, B.E., Knudsen, L.E., 2014. Association of maternal self-medication and over-the-counter analgesics for children. Pediatrics 133, e291–e298.

Jenssen, M.T.S., Brantsæter, A.L., Haugen, M., Meltzer, H.M., Larssen, T., Kvalem, H.E., Birgisdottir, B.E., Thomassen, Y., Ellingsen, D., Alexander, J., et al., 2012. Dietary mercury exposure in a population with a wide range of fish consumption—Self-capture of fish and regional differences are important determinants of mercury in blood. Sci. Total Environ. 439, 220–229.

Joas, R., Casteleyn, L., Biot, P., Kolossa-Gehring, M., Castano, A., Angerer, J., Schoeters, G., Sepai, O., Knudsen, L.E., Joas, A., et al., 2012. Harmonised human biomonitoring in Europe: activities towards an EU HBM framework. Int. J. Hyg. Environ. Health 215, 172–175.

Kolossa-Gehring, M., Becker, K., Conrad, A., Schroter-Kermani, C., Schulz, C., Seiwert, M., 2012. Environmental surveys, specimen bank and health related environmental monitoring in Germany. Int. J. Hyg. Environ. Health 215, 120–126.

Merlo, D.F., Agramunt, S., Anna, L., Besselink, H., Botsivali, M., Brady, N.J., Ceppi, M., Chatzi, L., Chen, B., Decordier, I., et al., 2014. Micronuclei in cord blood lymphocytes and associations with biomarkers of exposure to carcinogens and hormonally active factors, gene polymorphisms, and gene expression: the NewGeneris cohort. Environ Health Perspect 122, 193–200.

Modick, H., Schutze, A., Palmke, C., Weiss, T., Bruning, T., Koch, H.M., 2013. Rapid determination of N-acetyl-4-aminophenol (paracetamol) in urine by tandem mass spectrometry coupled with on-line clean-up by two dimensional turbulent flow/reversed phase liquid chromatography. J.Chromatogr. B Anal. Technol. Biomed. Life Sci. 925, 33–39.

Mørck, T.A., Erdmann, S.E., Long, M., Mathiesen, L., Nielsen, F., Siersma, V.D., Bonefeld-Jorgensen, E.C., Knudsen, L.E., 2014a. PCB concentrations and dioxin-like activity in blood samples from Danish school children and their mothers living in urban and rural areas. Basic Clin. Pharmacol. Toxicol. 115, 334–344.

Mørck, T.A., Nielsen, F., Nielsen, J.K.S, Siersma, V., Grandjean, P., Knudsen, L.E., 2014b. PFAS concentrations in plasma samples from Danish school children and their mothers. Chemosphere, http://authors.elsevier.com/sd/article/S0045653514008686 (in press).

Nikic, D., Stojanovic, D., Stankovic, A., 2005. Cadmium in urine of children and adults from industrial areas. Cent. Eur. J. Public Health 13, 149–152.

Nielsen, J.K.S., Modick, H., Mørck, T.A., Jensen, J.A., Nielsen, F., Koch, H., Knudsen, L.E., 2014. N-acetyl-4-aminophenol (paracetamol) in urine samples of 6-11-year-old Danish school children and their mothers. Int J Hyg Environ Health, http://www.sciencedirect.com/science/article/pii/S1438463914000601 (in press).

Olsson, I.M., Bensryd, I., Lundh, T., Ottosson, H., Skerfving, S., Oskarsson, A., 2002. Cadmium in blood and urine–impact of sex, age, dietary intake, iron status, and former smoking–association of renal effects. Environ. Health Perspect. 110, 1185–1190.

Passos, C.J.S., Mergler, D., Lemire, M., Fillion, M., Guimarães, J.R.D., 2007. Fish consumption and bioindicators of inorganic mercury exposure. Sci. Total Environ. 373, 68–76.

Pirard, C., Koppen, G., De Cremer, K., Van Overmeire, I., Govarts, E., Dewolf, M.C., Van De Mieroop, E., Aerts, D., Biot, P., Casteleyn, L., et al., 2014. Hair mercury and urinary cadmium levels in Belgian children and their mothers within the framework of the COPHES/DEMOCOPHES projects. Sci. Total Environ. 472, 730–740.

Richter, P.A., Bishop, E.E., Wang, J., Swahn, M.H., 2009. Tobacco smoke exposure and levels of urinary metals in the U.S. youth and adult population: the National Health and Nutrition Examination Survey (NHANES) 1999-2004. Int. J. Environ. Res. Public Health 6, 1930–1946.

Schindler, B.K., Esteban, M., Koch, H.M., Castano, A., Koslitz, S., Canas, A., Casteleyn, L., Kolossa-Gehring, M., Schwedler, G., Schoeters, G., et al., 2014. The European COPHES/DEMOCOPHES project: towards transnational comparability and reliability of human biomonitoring results. Int. J. Hyg. Environ. Health 217, 553–661.

Schoeters, G., Den Hond, E., Colles, A., Loots, I., Morrens, B., Keune, H., Bruckers, L., Nawrot, T., Sioen, I., De Coster, S., et al., 2012. Concept of the Flemish human biomonitoring programme. Int. J. Hyg. Environ. Health 215, 102–108.

Vande Loock, K., Fthenou, E., Decordier, I., Chalkiadaki, G., Keramarou, M., Plas, G., Roelants, M., Kleinjans, J., Chatzi, L., Merlo, F., et al., 2011. Maternal and